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The Commissioner of Patents

1 June 2004

Madam

IN THE MATTER OF International Patent Application No. PCT/AU03/00415
in the name of PROMICS PTY LIMITED
Entitled USE OF C5A RECEPTOR ANTAGONIST IN THE TREATMENT OF FIBROSIS
Our Ref: VS:CE:FP17710

We refer to the first Written Opinion dated 3 November 2003 issued by the International Preliminary Examining Authority in respect of this application, and offer the following comments in response to the objections of the Examiner, Ms Arati Sardana.

The Examiner has objected that the invention as defined in claims 1 to 4, 8 and 13 is not novel in the light of the disclosures of references D1 and D3 cited in the International Search Report, and that the invention as defined in claims 1 to 13 is not novel in the light of the disclosure of D2. The Examiner asserts that D1 and D3 disclose treating fibrotic conditions by administering a C5a receptor antagonist, and that D2 discloses treatment of these conditions by administering a C5 receptor antagonist of formula I as defined in the present specification.

D2, US Patent No. 4,692,511, discloses peptides which have activity as competitive antagonists of the C5a receptor, and are stated to have anti-inflammatory activity. The conditions listed at column 1 line 65 to column 2 line 7 as being amenable to treatment with these peptides include idiopathic pulmonary fibrosis. The citation discloses only *linear* peptides, which have as an essential core the tripeptide Asp-Gly-Ala or the tetrapeptides Try-Asp-Gly-Ala or Asp-Gly-Ala-Tyr. Amino and/or carboxyterminal extensions derived from the native human C5a sequence from amino acids 14 to 37 may be present. There is no disclosure or suggestion of any cyclic peptide or peptidomimetic compound, and in particular there is absolutely no suggestion of any compound of formula I according to the present application. Nor are there any experimental results at all to support the assertion that the compounds disclosed in the citation are effective for treatment of any fibrotic condition; in fact there is no evidence to show that any of the peptides disclosed in the citation has any activity at all as a C5a receptor antagonist. It is submitted that this citation is irrelevant to the invention.

D2, Australian Patent Application No 80926/98, lists a number of conditions for which the compounds of the invention disclosed therein could be useful, and fibrotic conditions are not

explicitly mentioned. A list of conditions is set out at pages 3 to 7, and this list includes ischaemic heart disease and myocardial infarct; however, no experimental evidence is presented to demonstrate that the compounds of the citation are actually effective in the treatment of any fibrotic condition.

D3, PCT Publication No. WO 02/14265, relates to bisubstituted urea derivatives, including spironolactone and pirfenidone which inhibit neutrophil responses to C5a. Again a list of conditions allegedly treatable using the compounds of the invention is provided; these include chronic obstructive pulmonary disease and cardiac infarction, but no experimental evidence is presented in relation to treatment of fibrosis. Not only are the compounds disclosed in this reference not peptides at all, their structure is completely unrelated to that of C5a receptors or G-protein-coupled receptors. Therefore the Examiner is incorrect in assuming that the compounds disclosed in this reference actually act as antagonists of C5a receptors. Again, this reference is irrelevant to the claimed invention.

It is respectfully submitted that none of the references represents an enabling disclosure, and that in particular D1 and D3 do not disclose cyclic peptides. Indeed, D3 does not disclose peptides at all, and although D1 discloses peptides, they are not cyclic peptides, and there is no evidence that any of the peptides disclosed in D3 has any activity at all.

Consequently it is respectfully submitted that D1 represents at best a mere paper anticipation, and therefore the invention as claimed is both novel and inventive in the light of this citation. D3 does disclose some activity of the compounds disclosed therein as antagonists of the action of C5a receptor, but merely mentions two conditions associated with fibrosis as being among a multitude of conditions potentially susceptible to treatment with C5a receptor antagonists. Since there is no disclosure or suggestion in this reference of any peptide antagonist, it is submitted that the invention as claimed is novel and inventive in the light of this citation. D2 does not explicitly mention fibrotic conditions, and presents no experimental evidence to show that the compounds disclosed therein are affective in the treatment of any fibrotic condition, or in any experimental model predictive of efficacy in the treatment of any fibrotic condition. Consequently it is submitted that the invention as claimed is also novel and inventive in the light of this citation.

The applicant's detailed comments regarding the pathogenesis of fibrosis are attached for the Examiner's convenient reference. These show that inflammation is *not* a necessary precursor of the development of fibrosis, and in particular that use of C5a antagonists to prevent or treat the fibrotic complications of hypertension is unexpected.

In particular, until the present invention there was nothing in the art to suggest that a low-molecular weight cyclic peptide antagonist of the C5a receptor might have any utility in the prevention or treatment of fibrosis.

While we do not concede that the Examiner's position is in any way justified, amendments to the claims are proposed in order to progress the application. The opportunity is also taken to

insert reference to the priority application and to rectify clerical errors which have come to notice.

We lodge herewith substitute pages 1, 5, 6, 30, 32 and 38 to 41, together with working copies indicating the nature and location of the proposed amendments.

Favourable reconsideration is requested.

Yours faithfully

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COMMENTS BY THE APPLICANT

Treatment of fibrosis with a C5a antagonist

Fibrosis develops when cells, such as fibroblasts, secrete fibronectin and collagen to produce a matrix of structural proteins. Fibrosis frequently follows inflammation, especially chronic inflammation, and is a normal part of the healing process. Without this mechanism many healing processes would be compromised (e.g. surgical wounds would not heal), but excessive deposition of fibrous tissue can also be undesirable, and can cause "disease" in its own right. Antiinflammatory drugs are commonly used to prevent the development of fibrosis which accompanies inflammation; one example of this is the use of corticosteroids and nonsteroidal antiinflammatory drugs (NSAIDs) to reduce intraocular scarring and loss of sight in patients with uveitis.

There are some diseases which are not classified as inflammatory, but which still have a marked component of fibrosis. In these diseases, an aetiological agent, other than an inflammatory agent, stimulates the production of structural proteins, such as collagen, in organs. Common examples of this type of pathology include diabetes mellitus and hypertension. It is believed that the stimulus for fibrosis in diabetes is damage to vascular endothelial cells resulting from elevated concentrations of glucose in the plasma. Vascular leakage as a result of an alteration in the physiology of the vascular endothelial cells can trigger expression of fibronectin and collagen in a variety of cells. In some cases, such as airway smooth muscle in asthma and vascular smooth muscle in hypertension and diabetes, these cells undergo an alteration in their phenotype before this stimulation of expression and secretion occurs. In hypertension, vascular endothelial damage, due in this case to elevated blood pressure, also appears to underlie the deposition of fibrous tissue in the walls of affected arteries. Although the end result is the same – vascular fibrosis – the mechanisms of this structural change are probably different in the two disease states, because rats treated with streptozotocin to induce Type I diabetes are hypotensive to normotensive. Deposition of collagen may also stimulate the influx and activation of inflammatory cells, such as macrophages, but this is a secondary process.

Endothelial cell damage alone may initiate fibrosis in tissues. In diabetes, this can occur in most organs, in particular heart, kidney, major blood vessels and retina, and in hypertension organ fibrosis is seen particularly in the heart, kidneys and large blood vessels. In clinical practice, antiinflammatory therapy is not used for the treatment of fibrosis associated with non-inflammatory causes. In fact, antiinflammatory therapy using non-steroidal anti-inflammatory drugs often exacerbates hypertension through the inhibition of the renal synthesis of prostaglandins. Thus, in these situations, standard antiinflammatory therapy may be contraindicated, and so teaches away from the potential application of C5a antagonists as anti-fibrotic therapy in non-inflammatory diseases.

It has been shown that the monocytes of newly-diagnosed Type I diabetics are activated (Josefsen K, Neilsen H, Lorentzen S, Damsbo P, Buschard K. Circulating monocytes are activated in newly-diagnosed type 1 diabetes mellitus patients. Clin Exp Immunol 1994 98:489-93). This study also showed that C5a chemotaxis was activated (Josefsen K, Neilsen H, Lorentzen S, Damsbo P, Buschard K. Circulating monocytes are activated in newly diagnosed type 1 diabetes mellitus patients. Clin Exp Immunol 1994 98:489-93). Although this suggests that the pathogenesis of type 1 diabetes may stimulate inflammatory events, the authors did not suggest that targeting of these events with specific pharmacological agents directed to C5a or its receptor could offer a treatment of fibrotic events in diabetes.

It should be noted that in both diabetes and hypertension inflammation does result from the primary pathological events, ie. elevated blood glucose in diabetes and the physical damage caused by high blood pressure in hypertension, but neither of these conditions can be regarded as an inflammatory disease because the underlying cause does not possess the elements of the classical immuno-inflammatory reaction. In particular, neither of these conditions can be effectively treated or prevented using conventional anti-inflammatory agents.

C5a is a recognised mediator of inflammation, especially in diseases of autoimmune origin or in severe chronic disease states. C5a has also been implicated in the pathology of hypertensive renal injury, with mice deficient in C5 showing less pathology than normal mice (Raij L, Dalmasso AP, Staley NA, Fish AJ. Renal injury in DOCA-salt hypertensive C5-sufficient and C5-deficient mice. Kidney Int. 1989 Oct;36(4):582-92.). These authors measured the degree of glomerular sclerosis, proteinuria and renal insufficiency (serum creatinine). In this study the authors have *not* drawn a link between this mechanism and possible treatment of the fibrosis of hypertension with specific anti-complement agents.

This demonstrates that the use of C5a antagonists in hypertension to prevent or treat potential fibrotic complications is novel and unexpected.

THERAPEUTIC METHOD

FIELD OF THE INVENTION

This application claims priority from Australian provisional patent application No. PS1606, filed on 8 April 2002.

This invention relates to the use of an antagonist of a G protein-coupled receptor in the prevention and/or treatment of fibrosis, such as the treatment of fibrosis associated with myocardial infarction, diabetes, or certain pulmonary conditions. In a preferred embodiment the antagonist is a C5a receptor antagonist, more preferably a cyclic peptide antagonist of the C5a receptor.

BACKGROUND OF THE INVENTION

All references, including any patents or patent applications, cited in this specification are hereby incorporated by reference. No admission is made that any reference constitutes prior art. The discussion of the references states what their authors assert, and the applicants reserve the right to challenge the accuracy and pertinency of the cited documents. It will be clearly understood that, although a number of prior art publications are referred to herein, this reference does not constitute an admission that any of these documents forms part of the common general knowledge in the art, in Australia or in any other country.

G protein-coupled receptors are prevalent throughout the human body, comprising approximately 60% of known cellular receptor types. They mediate signal transduction across the cell membrane for a very wide range of endogenous ligands and consequently participate in a diverse array of physiological and pathophysiological processes, including, but not limited to, those associated with cardiovascular, central and peripheral nervous system reproductive, metabolic, digestive, immunoinflammatory, and growth disorders, as well as other cell regulatory and proliferative disorders. Agents which selectively modulate

et al. 1997).

The effects of drug-induced and hypertension-induced pulmonary and renal fibrosis in animal models can be prevented or partially reversed by compounds which act by suppressing inflammatory events and down-regulating lung pro-collagen I over-expression (Iyer et al., 1999a,b).

We have shown that the administration of pirfenidone or spironolactone can prevent and partially reverse cardiac fibrosis and the increase in cardiac stiffness which occurs in streptozotocin-induced diabetes in rats (Miric G, et al., 2001) It is thought that pirfenidone acts by inhibiting increased TGF-β mRNA expression, allowing an increase in expression of metalloproteases which degrade the collagen I laid down during fibrosis. The mode of action of spironolactone is at present unknown. Spironolactone is a steroid analogue which is primarily used as a diuretic; pirfenidone (5-methyl-1-phenyl-2-(1H)-pyridone), an investigational compound being investigated as an anti-fibrotic agent in a number of indications.

It would be highly desirable to identify other therapeutically or prophylactically active agents for use in the treatment or prevention of fibrosis.

SUMMARY OF THE INVENTION

The overexpression or underregulation of a G-protein-coupled receptor, the C5a receptor, has been implicated in immune-system mediated events such as inflammation. Agents which influence C5a receptor activity, such as C5a receptor antagonists, have the potential to mediate inflammatory events, and may provide a means of therapeutic or prophylactic intervention, but have not previously been suggested as potential agents in the treatment or prevention of fibrosis.

We have now surprisingly found that a cyclic peptide with C5a receptor antagonist has the ability to

ameliorate cardiac fibrosis in an animal model of this condition.

According to a first aspect, the invention provides a method of prevention, treatment or alleviation of a fibrotic condition, comprising the step of administering an effective amount of an antagonist of a G protein-coupled receptor to a subject in need of such treatment.

The use of any compound having activity as an antagonist of a G protein-coupled receptor, and particularly as a C5a receptor antagonist, is contemplated, including but not limited to those disclosed in our earlier International patent applications No.PCT/AU98/00490 or No. PCT/AU02/01427 or in International patent applications No. PCT/US00/11187 by Neurogen Corporation and No. PCT/JP01/06902 by Welfide Corporation, or antibody antagonists such as those disclosed in PCT/US00/24219 or US patent No. 6355245. The entire disclosures of all of these specifications are incorporated herein by this cross-reference.

More preferably the C5a receptor antagonist is a peptide or a peptidometic compound, and more preferably is a cyclic peptide or a cyclic peptidometic compound. Even more preferably the compound is a cyclic peptide or a cyclic peptidometic compound of PCT/AU98/00490 or PCT/AU02/01427.

Still more preferably the antagonist is a compound which

(a) is an antagonist of a G protein-coupled receptor,
(b) has substantially no agonist activity, and
(c) is a cyclic peptide or peptidomimetic compound of formula I

Xylocaine to prevent airway spasm, the rats were intubated and a slow injection of bleomycin or saline control was completed. The rats were then rotated gently for about 1-2 minutes to allow the solution to diffuse evenly into both lungs (Christensen et al 2000). Rats were kept in the fume cupboard until totally recovered, and then monitored for up to 18 days. Body weight, food and water intake, and respiration were monitored daily.

Respiration was elevated as follows: Score 0, normal respiration; Score 1, increased rate of breathing; and Score 2, mouth open respiration. Rats were euthanased before the end of the experimental period, if they consistently lost more than 10% body weight for 48 hours, had Score 2 respiration or had Score 1 respiration for 48 hours.

At the end of this period the rats were killed by exsanguination under anaesthesia, so that the lungs were clear of blood. For each rat, the left lung was immediately frozen in liquid nitrogen and stored at $-20^{0}C$ for quantitative collagen analysis using hydroxyproline assay. The right lung was fully inflated and fixed with 10% formulated formalin by airway gravity fixation at a pressure of 30 cm water for 1 minute. Haematoxylin and eosin (H&E) and Picro Sirius Red (PR) staining for collagen were performed to assess collagen deposition in the lung. For quantitation of collagen stained with PR, polarized light images were converted to grey scale, and the total number of white pixels (specific for collagen) per image was determined as a percentage of the total pixel area. The procedure was applied to a total of four fields in the alveolar area and two fields in the peribronchial area and blood vessels per sample (Wang et al, 2000). The largest lobe of the right lung (from 4 lobes) in each rat was chosen. The data was analysed using the program "Sion Image".

Hydroxyproline assay was performed by the method

Table 1.
Lung weight and body weight in bleomycin-induced pulmonary fibrosis (7-9 days)

Condition	Left lung weight (g)	Body weight (g)	Ratio $x10^{-3}$
Normal	0.507 ± 0.003	240.6 ± 4.667	1.9 ± 0.36
Bleomycin	1.004 ± 0.04	226 ± 8.083	4.47 ± 0.46**
Bleomycin + PMX53	0.974 ± 0.132	228 ± 7.583	4.25 ± 1.07**

**: $P<0.001$, n=3, compared to normal rats.

Under the microscope, numbers of inflammatory cells, including PMNs, macrophages, lymphocytes etc. were observed in the alveolar spaces, with massive leakage of plasma and red blood cells; this is illustrated in Figure 13a. The size and number of type II AECs in the alveolar spaces was clearly increased, as shown in Figure 13b, while in normal lung, the type II AECs covered only 5 - 10% of the surface area of the alveoli, as shown in Figure 14.

There was no significant difference in histology between drug-treated and non-treated groups. Collagen deposition in bleomycin instillation lungs showed a significant increase compared to normal lungs ($P<0.01$, n=3); saline instillation lungs ($P<0.01$, n=3); and saline instillation with PMX53-treated lungs ($P<0.01$, n=3). However, there was no significant difference between the drug-treated group and non-treated group ($P>0.01$, n=4). These results are summarised in Figure 15.

2. Pulmonary fibrosis

Eighteen days after intra-tracheal instillation of bleomycin, the degree of oedema was reduced in bleomycin-instilled lungs, and the lung/body weight ratio did not

CLAIMS

1. A method of prevention, treatment or alleviation of a fibrotic condition, comprising the step of administering an effective amount of an antagonist of a C5a receptor to a subject in need of such treatment, in which the antagonist is a peptide or a peptidomimetic compound.

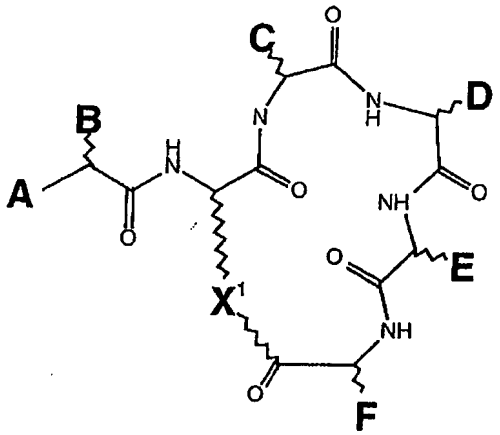
2. A method according to claim 1, in which the antagonist is a cyclic peptide or a cyclic peptidomimetic compound.

3. A method according to claim 1 or claim 2, in which the inhibitor is a compound which

a) is an antagonist of a G protein-coupled receptor,

b) has substantially no agonist activity, and

c) is a cyclic peptide or peptidomimetic compound of formula I



where A is H, alkyl, aryl, NH_2, NH-alkyl, $N(alkyl)_2$, NH-aryl, NH-acyl, NH-benzoyl, $NHSO_3$, $NHSO_2$-alkyl, $NHSO_2$-aryl, OH, O-alkyl, or O-aryl;

B is an alkyl, aryl, phenyl, benzyl, naphthyl or indole group, or the side chain of a D- or L-amino acid, but is not the side chain of glycine, D-phenylalanine, L-

homophenylalanine, L-tryptophan, L-homotryptophan, L-tyrosine, or L-homotyrosine;

C is the side chain of a D-, L- or homo-amino acid such as glycine, alanine, leucine, valine, proline, hydroxyproline, or thioproline, but is not the side chain of isoleucine, phenylalanine, or cyclohexylalanine;

D is the side chain of a neutral D-amino acid, but is the side chain of glycine or D-alanine, a bulky planar side chain, or a bulky charged side chain;

E is a bulky substituent, but is not the side chain of D-tryptophan, L-N-methyltryptophan, L-homophenylalanine, L-2-naphthyl L-tetrahydroisoquinoline, L-cyclohexylalanine, D-leucine, L-fluorenylalanine, or L-histidine;

F is the side chain of L-arginine, L-homoarginine, L-citrulline, or L-canavanine, or a bioisostere thereof; and

X is $-(CH_2)_nNH-$ or $(CH_2)_n-S-$, where n is an integer of from 1 to 4; $-(CH_2)_2O-$; $-(CH_2)_3O-$; $-(CH_2)_3-$; $-(CH_2)_4-$; $-CH_2COCHRNH-$; or $-CH_2-CHCOCHRNH-$, where R is the side chain of any common or uncommon amino acid.

4. A method according to claim 3, in which n is 2 or 3.

5. A method according to claim 3 or claim 4, in which A is an acetamide group, an aminomethyl group, or a substituted or unsubstituted sulphonamide group.

6. A method according to claim 5, in which A is a substituted sulphonamide, and the substituent is an alkyl chain of 1 to 6, or a phenyl or toluyl group.

7. A method according to claim 6, in which the substituent is an alkyl chain of 1 to 4 carbon atoms.

8. A method according to any one of claims 3 to 7, in which B is the side chain of L-phenylalanine or L-phenylglycine.

9. A method according to any one of claims 3 to 8, in which C is the side chain of glycine, alanine, leucine,

valine, proline, hydroxyproline, or thioproline.

10. A method according to any one of claims 3 to 9, in which D is the side chain of D-Leucine, D-homoleucine, D-cyclohexylalanine, D-homocyclohexylalanine, D-valine, D-norleucine, D-homo-norleucine, D-phenylalanine, D-tetrahydroisoquinoline, D-glutamine, D-glutamate, or D-tyrosine.

11. A method according to any one of claims 3 to 10, in which the antagonist is a compound which has antagonist activity against C5aR, and has no C5a agonist activity.

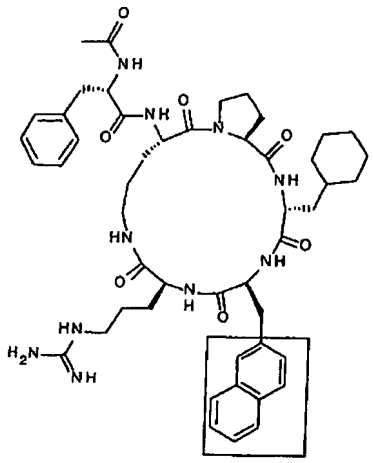
12. A method according to any one of claims 1 to 11, in which the inhibitor has potent antagonist activity at sub-micromolar concentrations.

13. A method according to any one of claims 1 to 12, in which the compound has a receptor affinity IC50<25μM, and an antagonist potency IC50<1μM.

14. A method according to any one of claims 1 to 13, in which the compound is selected from the group consisting of compounds **1** to **6**, **10** to **15**, **17**, **19**, **20**, **22**, **25**, **26**, **28**, **30**, **31**, **33** to **37**, **39** to **45**, **47** to **50**, **52** to **58** and **60** to **70** described in International patent application No.PCT/AU02/01427.

15. A method according to claim 14, in which the compound is AcF[OP-DCha-WR] (PMX53 compound **1**), AcF[OP-DPhe-WR] (compound **33**), AcF[OP-DCha-FR] (compound **60**) or AcF[OP-Dcha-WCit] (compound **45**).

16. A method according to claim 15, in which the compound is PMX53, having the formula



17. A method according to any one of claims 1 to 16, in which the fibrotic condition is selected from the group consisting of multiple sclerosis, proliferative vitroretinopathy, macular degeneration, scleroderma, sclerosing peritonitis, fibrosis arising from trauma, burns, chemotherapy, radiation, infection or surgery and fibrosis of the kidney, liver, heart or lungs, chronic hypertension and diabetes mellitus.

18. A method according to claim 17, in which the fibrotic condition is cardiac fibrosis or pulmonary fibrosis.

19. The use of a C5a receptor antagonist as defined in any one of claims 1 to 16 for the manufacture of a medicament for use in the treatment of a fibrotic condition.

20. A use according to claim 19, in which the fibrotic disorder is selected from the group consisting of multiple sclerosis, proliferative vitroretinopathy, macular degeneration, scleroderma, sclerosing peritonitis, fibrosis arising from trauma, burns, chemotherapy, radiation, infection or surgery and fibrosis of the kidney, liver, heart or lungs, chronic hypertension and diabetes mellitus.

21. A use according to claim 20, in which the fibrotic condition is cardiac fibrosis or pulmonary fibrosis.